Synthesis of Conformationally Restricted and Optically Pure Analogues of Serine-Proline Dipeptide via Aldol Condensation

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Abstract: Parallel synthesis of a small library of hydroxy functionalized bicyclic lactams with optically pure mimicking dipeptide Pro-Ser has been accomplished via aldol condensation of aldehyde 9 with N-BocGlyCO₂Et. The configurations of eight scaffolds were established unambiguously by NMR spectroscopy.

Key words: lactams, bicyclic compounds, peptidomimetics, asymmetric synthesis, aldol reaction

The poor receptor subtype selectivity, poor biostability and unfavorable absorption properties that often accompany therapeutically relevant peptides has generated a considerable amount of interest in the design of high affinity and selective peptidomimetics.1 A common feature of their design makes use of conformationally constrained structures such as fused bicycles. It is very well known that the inherent structural rigidity of such molecules hampers the rotation around the C–N and C–C bonds present in the natural peptides, and in some cases, leads to a favorite preorganization of the binding sites, which in turn induces a secondary structure into the peptide backbone.2

In the last decade we have assisted the flourishing development of elegant synthetic routes for the preparation of peptide mimics based on bicyclic structures.3 However, there has not been so much effort devoted to the preparation of similar scaffolds bearing functional groups suitable for the synthesis of more complex molecules, such as mimics of peptide conjugates or glycopeptides.4

Thus, in connection with our constant interest on developing new synthetic routes for the preparation of scaffolds by means of combinatorial technology, we wish to report herein on a parallel synthesis of hydroxyl functionalized fused bicyclic lactams mimicking the Ser-Pro dipeptide, i.e. 1–8 (Figure 1). These lactams, characterized by three different points of derivatization, may be used as scaffolds in parallel/combinatorial syntheses.

Recently, we have reported the enantioselective syntheses of a library of amino acid scaffolds, based on azaoxobicyclic structures.3c The versatility of our synthetic route relies on the key intermediate aldehydes 9a,b (Scheme 1), which can be conveniently prepared in a multigram scale from pyroglutamic acid.3c Thus, aldehydes 9a or b were condensed at low temperature (–78 °C, THF) with the commercially available N-Boc-glycine ethyl ester, affording an almost equimolar mixture of the four diastereoisomers respectively (10a–d or 11a–d), in an overall yield of around 80% (Scheme 1). The diastereomeric ratios were determined by HPLC on the crude materials and are reported in Table 1. Despite that Zn-chelated enolates lead to a poor diastereoselectivity, the presence of ZnCl₂ is crucial.5 In fact, using Li-enolates (2...
equiv of LDA) the yields decreased to 32% in the case of aldehyde 9a and to 25% in the case of 9b. Unreacted starting material was mostly recovered accompanied by a limited amount of unidentified by-products. Adding 1 mol equivalent of ZnCl₂ to the Li-enolate solution did not affect the reaction course (same yield and selectivity).

Unreacted aldehyde 9a and 9b. Reactants due to the existence of which by NMR analysis, appear as a mixture of conformers. Purification of the aldol mixture by flash chromatography allowed the separation of only two out of the four diastereoisomers, i.e. 10b and 10c or 11a and 11d, respectively, which by NMR analysis, appear as a mixture of conformers due to the existence of cis- and trans-isomers about the proline N-Cbz amide bond. The remaining diasteromeric mixtures, i.e. 10a + d or 11b + c, were submitted without further separation to the following steps of the synthesis. These consisted in the cleavage of Cbz amino protecting group, which was quantitatively removed by standard catalytic hydrogenation on Pd/C, followed by thermally induced intramolecular reaction of the free amino group with the ethyl ester, which lead to the mixtures of lactams, i.e. 2 and 4 or 5 and 7. At this stage the diastereoisomers could be easily separated by chromatography. Similarly, the remaining aldol adducts (10b,c and 11a,d) were submitted to the same synthetic sequence respectively the scaffolds 1, 3, 6 and 8. The overall yields of deprotection and thermal cyclization reactions are summarized in Table 2. The temperature in the cyclization reaction was found to be crucial, the best yields for the cyclization reaction were obtained using toluene (90 °C), whereas the yields decreased on using methanol and N,N-diisopropylethylamine (70 °C).

Table 2 Yields of Lactams 1–8 Over Two Steps: Deprotection and Cyclization

<table>
<thead>
<tr>
<th>Entry</th>
<th>Substrate</th>
<th>Toluene/TEA Yield (%)</th>
<th>MeOH/DIPEA Yield (%)</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10a</td>
<td>85</td>
<td>73</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>10b</td>
<td>73</td>
<td>55</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>10c</td>
<td>75</td>
<td>60</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>10d</td>
<td>89</td>
<td>76</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>11a</td>
<td>89</td>
<td>71</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>11b</td>
<td>80</td>
<td>48</td>
<td>7</td>
</tr>
<tr>
<td>7</td>
<td>11c</td>
<td>86</td>
<td>68</td>
<td>5</td>
</tr>
<tr>
<td>8</td>
<td>11d</td>
<td>86</td>
<td>48</td>
<td>6</td>
</tr>
</tbody>
</table>

Table 1 Aldol Reactions of N-Boc-glycine Ethyl Esters with Aldehyde 9a or 9b

<table>
<thead>
<tr>
<th>Entry</th>
<th>Aldehyde</th>
<th>ZnCl₂ (Equiv)</th>
<th>Products</th>
<th>dr*</th>
<th>Yieldb (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9a</td>
<td>2.1</td>
<td>10a.b.c.d</td>
<td>1.1:1.3:1:0.0.9</td>
<td>77</td>
</tr>
<tr>
<td>2</td>
<td>9a</td>
<td>–</td>
<td>10a.b.c.d</td>
<td>1.0:1.0:1.0:0.5</td>
<td>32</td>
</tr>
<tr>
<td>3</td>
<td>9b</td>
<td>2.1</td>
<td>11a.b.c.d</td>
<td>1.2:1.4:1.1:1.0</td>
<td>78</td>
</tr>
<tr>
<td>4</td>
<td>9b</td>
<td>–</td>
<td>11a.b.c.d</td>
<td>1.0:1.0:1.2:0.4</td>
<td>25</td>
</tr>
</tbody>
</table>

* Based on HPLC of the crude reaction mixtures, column LiChro-CART 250-4, (R,R)-Whelk-O1 (5 μm), hexane–propan-2-ol (8:2).

** Yields refer to the mixtures of diastereoisomers.

Finally, the configurations of the eight lactams were determined unambiguously by NMR spectroscopy using the following procedure. The cis relation of the newly formed stereocenters in compounds 1, 5 and 6 (Figure 1) was evidenced by the presence of NOEs between the protons H-3 and H-4 (Figure 1). This NOE was absent in the trans series 3, 4, 7 and 8. Furthermore, the presence of NOE between the proton H-3 and H-6 in compounds 1 and 6 allowed to unequivocally assign the configuration of compounds 1 and 6 as 3R,4R,6S,9R and 3S,4S,6R,9R, respectively, since the stereochemistry at C-6 is known and conserved during the synthesis. The presence of NOE between H-3 and H-6 in compound 7 and between H-4 and H-6 in compound 8 allowed to unequivocally assign the configuration of compound 7 and 8 as 3S,4R,6R,9R and 3R,4S,6R,9R, respectively. In the case of scaffolds 2–4, due to the overlap of the relevant proton signals of their spectra, the analyses were carried out on the corresponding O-acetyl derivatives, i.e. 12–14, showing a more suitable dispersion of the chemical shifts. The acetates were obtained by treatment with Ac₂O and pyridine in 60–80% yields (Scheme 1).
For compound 12, NOEs between H-3 and H-4 and between the methyl of the acetyl group and H-6 were observed unequivocally assigning the relative configuration as 35,43s,65S,9R. In the case of 13 the stereochemistry was determined observing the NOE between H-4 and H6 and the NH group with H-4.

In conclusion a parallel synthesis of a small library of optically pure scaffolds mimicking the dipeptide Ser-Pro has been achieved by mean of aldol reactions on aldehyde 9a or 9b. All reaction steps proceed with good yields using common reagents and combinatorial technology apparatus.

All chemicals and solvents were of reagent grade and were used without further purification. Solvents were dried by standard procedures, and reactions requiring anhydrous conditions were performed under 

Aldol Condensation; General Procedure A

To a solution of anhyd 1-Pr,NH (2.5 mL, 17.69 mmol) in anhyd THF (100 mL) at 0 °C and under 

NMR (200 MHz, CDCl3): δ (mixture of conformers) = 1.30 (m, 12 H, CH2,CH2O, Boc), 1.44 (s, 9 H, CO2-t-Bu), 1.60–2.40 (m, 6 H), 3.70–4.40 (6 H, CHCO-t-Bu, CH2,CH2O, OH, CHNcbz, CHOH), 4.95 (m, 1 H, CHNHBoc), 5.05, 5.12 (2 d, 2 H, J = 13.0 Hz, CH2Ph), 5.53 (d, 1 H, J = 9.0 Hz, NHBoc), 7.25–7.40 (5 H, C6H5).

13C NMR (50.3 MHz, CDCl3): δ (mixture of conformers) = 171.2, 170.4, 156.8, 155.4, 153.3, 138.0, 127.8, 79.7, 69.4, 67.5, 66.9, 61.1, 60.6, 60.1, 57.8, 55.0, 53.4, 52.5, 49.1, 48.4, 40.3, 29.5, 28.6, 28.2, 27.8, 27.6, 27.3, 14.1.

MS (FAB+): m/z calculated for C33H31N2O5: 552.29; found: 551.00.

Calcd. For C33H31N2O5: C, 61.07; H, 7.69; N, 5.17. Found: C, 61.08; H, 7.58; N, 5.06.

Aldehydes 9a-c were subjected to the aldol condensation using the parallel synthesis. 

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1^1 C NMR (50.3 MHz, CDCl₃): δ (mixture of conformers) = 171.5, 170.8, 156.1, 155.3, 135.8, 128.2, 127.9, 127.6, 81.2, 79.3, 68.7, 67.5, 60.9, 60.1, 57.8, 55.4, 38.6, 30.3, 39.4, 28.7, 28.1, 27.6, 14.0.

MS (FAB⁺): calc'd for C₃₉H₃₈N₂O₂; m/z found: 559.


**Cyclization in Toluene; General Procedure D**

A solution of aldol 10 or 11 (0.4545 g, 0.82 mmol) in MeOH (8.2 mL) containing a catalytic amount of 10% Pd/C was stirred overnight under H₂. The catalyst was then removed through the Celite pad, the solvent was evaporated under reduced pressure and the crude was used without further purification.

**Catalytic Hydrogenation; General Procedure B**

A solution of aldol 10 or 11 (0.4545 g, 0.82 mmol) in MeOH (14 mL), i-Pr₂NEt (440 µL) was refluxed for 4 days. Thereafter, the solvent was removed under reduced pressure and the crude was purified by flash chromatography (hexane–EtOAc, 3:7) to afford lactams 1–8.

**Cyclization in Toluene; General Procedure D**

The product of catalytic hydrogenation (0.82 mmol) was dissolved in toluene (42 mL), i-Pr₂NEt (440 µL, 5.48 mmol) and the solution was refluxed for 4 days. Thereafter, the solvent was removed under reduced pressure and the crude was purified by flash chromatography (hexane–EtOAc, 3:7) to afford lactams 1–8.

**(3R,4R,6R,9S)-1-Aza-9-tert-butoxycarbonyl-3-tert-butoxycarbonyl-bicyclo[4.3.0]nonane-4-carboxylic acid (43.0)monanone (3)**

Mp 168–170 °C; [α]D<sup>25</sup> = −57.9 (c = 1.02, CHCl₃).

**(3R,4R,6R,9S)-1-Aza-9-tert-butoxycarbonyl-3-tert-butoxycarbonyl-bicyclo[4.3.0]nonane-4-carboxylic acid (43.0)monanone (4)**

Mp 137–139 °C; [α]D<sup>25</sup> = −110.3 (c = 0.72, CHCl₃).

**(3R,4R,6R,9S)-1-Aza-9-tert-butoxycarbonyl-3-tert-butoxycarbonyl-bicyclo[4.3.0]nonane-4-carboxylic acid (43.0)monanone (5)**

Mp 56–58 °C; [α]D<sup>25</sup> = −26.1 (c = 1.05, CHCl₃).

**(3R,4R,6R,9S)-1-Aza-9-tert-butoxycarbonyl-3-tert-butoxycarbonyl-bicyclo[4.3.0]nonane-4-carboxylic acid (43.0)monanone (6)**

Mp 49–50 °C; [α]D<sup>25</sup> = −0.2 (c = 1.03, CHCl₃).

**(3R,4R,6R,9S)-1-Aza-9-tert-butoxycarbonyl-3-tert-butoxycarbonyl-bicyclo[4.3.0]nonane-4-carboxylic acid (43.0)monanone (7)**

Mp 62–63 °C; [α]D<sup>25</sup> = −67.1 (c = 1.03, CHCl₃).

**(3R,4R,6R,9S)-1-Aza-9-tert-butoxycarbonyl-3-tert-butoxycarbonyl-bicyclo[4.3.0]nonane-4-carboxylic acid (43.0)monanone (8)**

Mp 85–87 °C; [α]D<sup>25</sup> = −94.2 (c = 1.03, CHCl₃).

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(3S,4R,6R,9S)-1-Aza-9-tert-butoxycarbonyl-3-tert-butoxycarboxy-bicyclo[4.3.0]nonane-4-hydroxy-2-oxobicyclo[4.3.0]nonane (7)

Mp 111–113 ºC; [α]D 25 +40.0 (c = 1.05, CHCl3).

1H NMR (200 MHz, CDCl3): δ = 1.48 (s, 18 H, CO2-Bu, Boc), 1.60–2.35 (m, 6 H), 3.90 (m, 2 H, CHN, CH2NHBOc), 4.15 (m, 1 H, CHO), 4.35 (dd, 1 H, J = 8.4, <1 Hz, CHCO2-Bu), 5.35 (br s, 1 H, OH), 6.20 (br s, 1 H, NH).

13C NMR (50.3 MHz, CDCl3): δ = 170.3, 165.6, 95.3, 81.7, 80.4, 73.7, 59.9, 59.6, 54.4, 38.3, 30.6, 29.0, 28.1, 27.8.

MS (FAB+): m/z calcld for C31H49NO7: 530.21; found: 531.

Anal. Calcd for C31H49NO7: C, 58.36; H, 8.16; N, 7.56. Found: C, 58.43; H, 8.18; N, 7.54.

(3R,4S,6R,9S)-1-Aza-9-tert-butoxycarbonyl-3-tert-butoxycarbonyl-4-hydroxy-2-oxobicyclo[4.3.0]nonane (8)

Mp 67–70 ºC; [α]D 25 +55.0 (c = 1.03, CHCl3).

1H NMR (200 MHz, CDCl3): δ = 1.48 (s, 18 H, CO2-Bu, Boc), 1.70–2.10 (m, 5 H), 2.41 (m, 1 H), 3.55 (m, 1 H, CHN), 3.88 (dd, 1 H, J = 9.2, 3.6 Hz, CH2NHBOc), 4.05 (m, 1 H, CHO), 4.30 (dd, 1 H, J = 9.8, <1 Hz, CHCO2-Bu), 5.61 (br s, 2 H, OH, NH).

13C NMR (50.3 MHz, CDCl3): δ = 170.2, 166.0, 158.4, 81.6, 80.9, 71.4, 60.2, 59.1, 56.5, 36.0, 29.6, 28.5, 27.8, 25.7.

MS (FAB+): m/z calcld for C32H51NO7: 370.21; found: 371.

Anal. Calcd for C32H51NO7: C, 58.36; H, 8.16; N, 7.56. Found: C, 58.50; H, 8.18; N, 7.54.

Acetylation: General Procedure E

Lactams 2–4 (0.037 g, 0.1 mmol) were dissolved in pyridine (40 µl, 0.5 mmol), Ac2O (47 µl, 0.5 mmol) and was added and the solution was stirred at r.t. After 4 h, H2O (1 mL) was added and the mixture was extracted with EtOAc (3 × 1 mL). The combined organic layers were dried (Na2SO4), filtered and the solvent was evaporated. The crude was purified by flash chromatography (hexane–EtOAc, 4:6) to afford 12–14 (yield: 70–83%).

(3S,4S,6S,9S)-4-Acetoxy-1-aza-9-tert-butoxycarbonyl-3-tert-butoxycarbonyl-4-hydroxy-2-oxobicyclo[4.3.0]nonane (12)

[α]D 25 =−40.3 (c = 1.43, CHCl3).

1H NMR (200 MHz, CDCl3): δ = 1.47, 1.49 (2 s, 18 H, CO2-Bu, Boc), 1.50–2.45 (m, 6 H), 2.12 (s, 3 H, CH3CO), 3.81 (m, 1 H, CHN), 4.31 (m, 2 H, CHCO2-Bu, CH2NHBOc), 5.17 (d, 1 H, J = 8.5 Hz, NH), 5.49 (m, 1 H, CH2OAc).

13C NMR (50.3 MHz, CDCl3): δ = 170.9, 170.1, 165.5, 104.8, 81.2, 79.9, 69.5, 58.5, 54.8, 53.6, 32.3, 31.1, 28.2, 27.9, 27.8, 20.9.

MS (FAB+): m/z calcld for C33H53NO8: 542.22; found: 413.

Anal. Calcd for C33H53NO8: C, 58.24; H, 7.82; N, 6.79. Found: C, 58.20; H, 7.83; N, 6.77.

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References


